Plant Archives Vol. 19 No. 2, 2019 pp. 3805-3810 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF URANIUM ON SEED GERMINATION OF *CLEOME AMBLYOCARPA* BARR. & MURB.

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Abstract

Uranium raises particular attention, due to the deplorable situation in the environment, it is responsible for radioactive pollution of the soil and it poses serious problems for the human health and the protection of ecosystems through its radiotoxicity and chemo-toxicity. *Cleome amblyocarpa* Barr. & Murb*,* is a species representative of the Algerian Saharan ecosystem, is an annual herbaceous plant of the *Caparidaceae* family. The objective of this work is to study the effect of uranium in the form of hydrated hexa uranyl nitrate $[UO_2(NO_3)_2\text{-}6H_2O]$ on the germination rate and speed of *Cleome amblyocarpa* Barr. & Murb, as well as the growth of the radicle and tigelle. The seeds of *Cleome amblyocarpa* Barr. & Murb, have been put to germinate in the dark in an oven set at 26°C, soaked in concentrations of uranium at 0.50,100,150,200,250 and 300 ppm at five repetitions for each treatment. The results obtained show that the germination rate of the plant seeds increases at the applied rates of 100, 150 and 200 ppm, whereas there is a decrease in the germination rate at higher applied concentration (250 ppm and 300 ppm). The length of the aerial parts of the seedlings decreases as the applied uranium doses increase. Concentration of uranium at 50, and 100 ppm, stimulate radicle elongation of seedlings relative to the control. However, the radicle elongation of seedlings gradually decreased at concentrations of 150, 200, 250 and 300 ppm respectively. These results indicate that *Cleome amblyocarpa* Barr. & Murb tolerates the metallic stress caused by uranium during the germinative phase. These results can be used as a basis for studying the effect of uranium on *Cleome amblyocarpa* planted in uranium-contaminated soil, which can be used as a phytoremediator species.

Key words**:** Uranium, *Cleome amblyocarpa* Barr. & Murb, Germination, Stress.

Introduction

The intensification of industrial and agricultural activities as well as the increase in population (Gold, 2002) are at the origin of a contamination of our environment by heavy metals and organic pollutants (Vavasseur *et al.,* 2003).

Uranium (U) is a natural radionuclide that exists in the isotope forms of U^{238} (99.07%), U^{235} (0.72%) and U^{234} (0.01%), which is widely used in industry, agriculture, medical treatment, national defense and scientific research (Tripathi *et al*., 2013). The International Commission on Radiological Protection (ICRP) has postulated that man is the most radiosensitive being and that protecting him therefore means not endangering other species (ICRP, 1991).

In addition to conventional physical and chemical methods, the development of effective biological technology to control radioactive pollution has become an important subject worldwide (Kim *et al.*, 2013). One of them, phytoremediation, exploits the properties of some plants to accumulate large quantities of heavy metals (Prabha *et al*., 2007). Phytoremediation is a plant biotechnology with ecological and financial advantages. phytoremediation is a pollution control technology that appears to be effective for a wide range of organic and inorganic pollutants. It can be used on solid, liquid or gaseous substrates (Pilon-Smits, 2005). Some species are tolerant and more effective at absorbing pollutants such as uranium.

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Cleome amblyocarpa Barr. & Murb is a spontaneous Saharan species that adapts to the climatic and edaphic conditions of the desert environment. It is a perennial plant, of the *Capparidaceae* family branched, yellowish green, 10 to 40cm high, with odour foul and unpleasant. Straightened rods, trifoliolate leaves. Leaflets lanceolate, flowers purple and large number of fruits in elongated and hairy capsules (Ozenda, 1991, Quezel and Santa, 1963). The "Netile" is considered by nomads as a toxic plant causing nervous disorders (Le Floc'h, 1983). This herb is also a medicinal plant used to ease pain. When mixed with *Juniperus phoenicia, Hammada scoparium* it treats headaches and when mixed with *Artemisia herba-alba* it becomes a treatment for nausea, gastralgia, vomiting and colic (Molino, 2005). The anti-inflammatory activity of *C. amblyocarpa* leaf extract, observed *in vivo* as well as *in vitro*, could be due to its high flavonoid content (19%) (Bouriche *et al.,* 2003, Bouriche and Arnhold, 2010).

The plant's response to uranium-induced stress can be expressed in seed germination, seedling growth and enzyme activity (Pereira *et al*., 2009). Seed germination and root elongation are two important physiological indicators in the early stage of plant growth to assess plant tolerance (Jing *et al*., 2018). There is little work that has studied the impact of uranium on germination. To this end, the objective of this work is to evaluate the metallic stress caused by uranium in the form of hydrated hexa uranyl nitrate ($UO_2(NO_3)_2$-$6H_2O$) on the rate and speed of germination of seeds of this species, as well as the growth of the radicle and tigelle.

Materials and Methods

Plant material

Cleome amblyocarpa seeds: The seeds used have been harvested since June 2018, from a site traced by a national road RN6, in EL BAYADH district located at (33°54′54.21″N, 0°12′20′59″E, South-East Algeria), in the high steppe plains of South-West Algeria (Fig. 1).

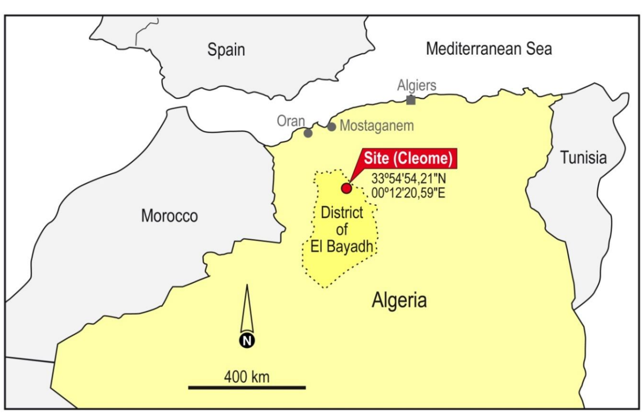

Fig. 1: Seed collection site.

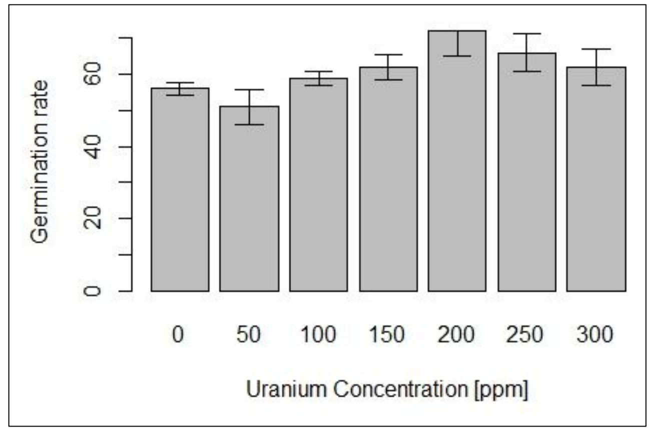

Fig. 2: Effect of uranium on seed germination of *Cleome amblyocarpa*.

Seed treatment: Seed treatment is carried out during the month of October 2018 at the biodiversity and soil and water conservation laboratory of Mostaganem University (UMAB), Algeria. The seeds are disinfected beforehand by a three-minute passage in concentrated sodium hypochlorite solution at 8°. They are then washed thoroughly with distilled water and dried on absorbent paper. Before germination, the seeds are placed in plastic petri dishes (20 seeds per dish) at seven U concentration (0, 50, 100, 150, 200, 250 and 300 ppm). A volume of 10 ml of hydrated hexa uranyl nitrate ($UO_2(NO_3)2$-$6H_2O$) was applied with five repetitions for each treatment.

Germination of seeds: Cleome seeds have been put to germinate in the dark in an oven set at 26°C, the duration of germination is fifteen (15) days, each day the seeds are removed, counted to determine the seeds having germinated, then returned to germinate again in the oven. The radicle breakthrough criterion was adopted to evaluate seed germination. A seed is considered germinated when the white radicle fate out of the integument. These counts are repeated daily during fifteen (15) days of germination.

Parameters analyzed

Final germination rate: A seed was considered germinated when the radicle pierced the envelope and became visible to the naked eye, as defined by Come (1970). And the final germination rate was calculated according to the following ratio:

TG (%) = Gx / Gt × 100

Where, TG: Final germination rate, Gx: number of germinated seeds, Gt: total number of seeds put to germinate.

Kinetics of germination: It is expressed as a daily percentage of seeds germinated in relation to the total number of seeds per petri dish (%) (Mazliak, 1982), during fifteen (15) days. Indeed, the germination rate is calculated according to the following formula:

TG (%) = *Gx* / *Gt* × 100

Where: TG: Average germination rate in (%), Gx: Number of germinated seeds, Gt: Total number of seeds put to germinate.

Radicle and tigelle length: It was measured with a graduated ruler, of six seeds per box every two days for fifteen (15) days to assess the plant's growth in response to stress. Measurements of this parameter are made from the 3rd day of the experiment until the end of the test (15th day).

Statistical analyses: The results obtained were statistically analyzed using software R version 3.5.2 (2018-12-20), an ANOVA analysis of variance is carried out to study the possible effect of uranium on the seed germination.

Results and Discussion

Effect of uranium on seed germination: The results show that all *Cleome amblyocarba* seeds tested germinate with a rate greater than 40% for all doses. The control seeds and treated seeds start to germinate from the 3rd day. There is an insignificant proportional increase or the probability is equal to 0.07 (P>0.05) in the germination rate with values of 59, 62 and 72%, respectively with the applied doses of U at 100.150 and 200 ppm. There was a decrease in the germination rate for the 250 and 300 ppm of U to 66% and 62% respectively. Constant germination is noted until the 15th day (Fig. 2).

Effect of uranium on the kinetics of the germination rate of *Cleome amblyocarpa*: relatively to the germination kinetics of *Cleome amblyocarpa* seeds treated at 100, 150 and 200 ppm of U (Fig. 3), germination becomes faster with the number of days and the final germination rate is higher than *Cleome amblyocarpa* seeds, treated

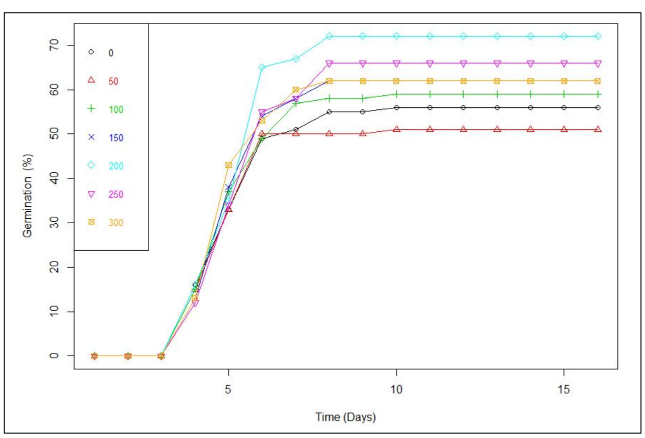

Fig. 3: Effect of uranium on the germination kinetics of *Cleome amblyocarpa* seeds.

at 50 ppm U doses and the control, or germination kinetics is slower. Germination begins on the 3rd day and decreases significantly from 200 ppm U or the probability is 0.04 (P<0.05).

The effect of U at low concentrations of 100, 150 and 200 ppm on the germination capacity of *Cleome amblyocarpa* is reflected in a stimulation of the germination rate which is slightly affected from 250 and 300 ppm of U. Studies show that low uranium concentrations promote the germination of tomato seeds, cucumber, kohlrabi, radish, cabbage, and spinach. And a high concentration of uranium inhibits the germination of the seeds of some vegetables. (Jing *et al.,* 2018).

The significant inhibitory effect of U on the germination rate of tomato, cucumber, kohlrabi, radish, cabbage and spinach is observed at 320, 1280, 640, 640, 320 and 320 mg/kg (p\0.05), respectively. Seed mortality rates of tomato, kohlrabi, cabbage, and spinach is 100% when the U concentration soil reaches or above 1280, 2560, 2560 and 2560 mg/kg, respectively. However, cucumber and radish seeds can germinate even under the maximum concentration of 2560 mg/kg (Jing *et al.,* 2018). The higher the germination rate and the faster the growth is, the lower the U in the tissue will be. There are three possible explanations:

1. Low concentration of U can stimulate the activity of some enzymes that promote the growth of seedlings, and then promote seed germination and seedling growth.
2. Because of the low concentration of U, the photosynthetic pigment content and net photosynthetic rate increased, the assimilation ability of seedlings was enhanced and the seed germination rate and root length were increased.
3. The low concentration of U treatment reduces the transpiration efficiency of the plant, thus improving the water use efficiency of the seeds and make it sprout quickly (Jagetiya and Purohit, 2006).

Other research that have studied the impact of heavy metals such as uranium on germination (rate and speed of germination, seedling viability, etc.) from 100ppm uranium in soil, the germination rate of corn seeds (*Zea mays*) is reduced, this reduction reaches about 35% (compared to the control) to 1000 ppm. Thus, the survival rate of germinated corn seeds (*Zea mays*) is also slightly affected from 500 ppm uranium (Stojanovic *et al.,* 2010). Other work has reported that bean germination (*Phaseolus vulgaris*) is not affected at a soil uranium content of 1000 ppm (Sheppard *et al.,* 1992). The overall toxicity thresholds for uranium for the different species

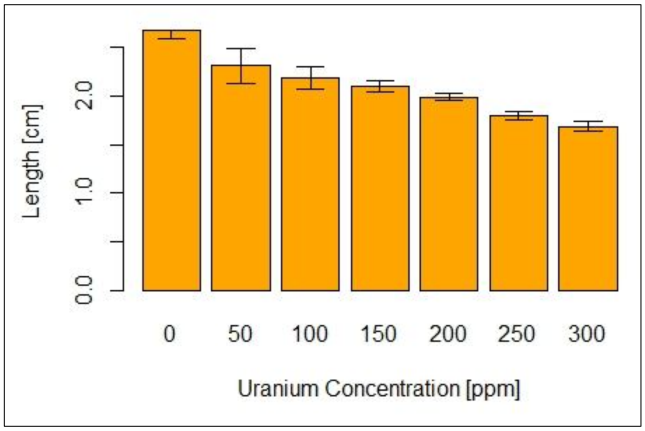

Fig. 4: Effect of uranium on the tigelles length of *Cleome amblyocarpa* .

tested are relatively high and higher than for other metals, such as arsenic or zinc (3 to 8 times) (Sheppard *et al.,* 1992). On the other hand, Sheppard and Thibault (1992) concluded an absence of effect below 300 ppm of uranium in soils for 5 plant species and 11 soils tested. Early studies, dating back to the beginning of the last century, have shown that very low uranium concentrations can stimulate seed germination (Stoklasa & Penkava, 1928). Aery and Jain (1997) have highlighted a slight stimulation of germination (earlier germination) of wheat seeds (*Triticum*) in the presence of low levels of uranyl nitrate.

Effect of uranium on the tigelles length of *Cleome amblyocarpa*: Compared to the control, where tigelles length is estimated at 2.67 cm, *Cleome amblyocarpa* seedlings show a decrease in tigelles length; after 15 days of exposure to different concentrations of U at: 50, 100, 150, 200, 250 and 300 ppm with 2.31, 2.19cm, 2.10, 1.99, 1.80 and 1.69cm respectively. These results show that metallic stress exerts an effect on the growth of *Cleome amblyocarpa* seedlings, resulting in a highly significant decrease ($P<0.001$) in the length of the aerial part as a function of the increase in U concentrations (Fig. 4).

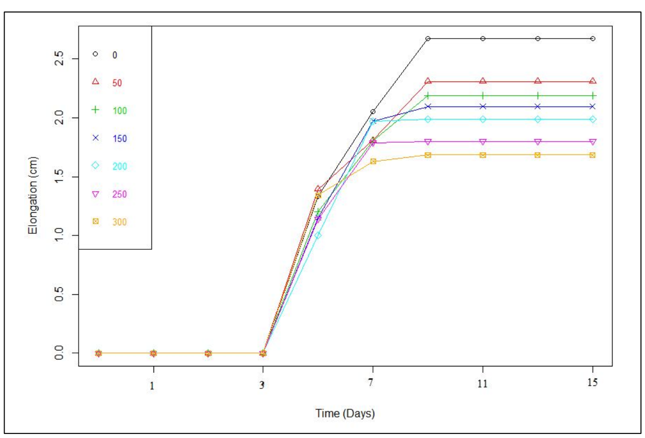

Fig. 5: Effect of uranium on the kinetics of the tigelles length of *Cleome amblyocarpa.*

Effect of uranium on the kinetics of the tigelles length of *Cleome amblyocarpa*: According to the results shown in fig. 5, *Cleome amblyocarpa* seedlings treated at different concentrations (0 to 300 ppm) of U for 15 days have a slower aerial elongation kinetics by concentration increment, the highest value occuring at 0 ppm. The lengthening begins on the 15th day and continues until the 9th day. Statistical analysis shows that the length of *Cleome amblyocarpa* tigelles decreases significantly or the probability is 0.04 ($P< 0.05$) as a function of the increase in U concentrations (Fig. 5).

The effect of U at low concentrations affects the growth of the aerial parts of *Cleome amblyocarpa* seedlings, resulting in a decrease in tigelle length. Horemans *et al.*, (2010) showed that during a 3-days exposure, uranium caused a decrease in fresh biomass in *A. thaliana* with an EC50 of 66 µM. Toxicity symptoms reported by Murthy *et al*., (1984) in 4-week-old soybean (Glycine max) plants resulted in chlorosis and early leaf abscission. From a high contamination (42 mg/L of uranium oxide), a wide diffusion of tissue necrosis occurred. In the case of high soil contamination (greater than 200 ppm), Greek horn (*Hibiscus esculentus*) plants show chetive growth, decreased green color, reduced stem and leaf size and delayed flowering (Singh *et al.,* 2005). In another study, chilli pepper (*Capsicum annuum*) and cucumber (*Cucumis sativus*) were found to be highly affected by soil uranium levels above 263 ppm, beyond that, plants paled and died after a few weeks (Unak *et al.,* 2007). Above 100 ppm uranium in soil, the dry matter yield of maize (*Zea mays*) decreases significantly, reaching about half the control yield (without uranium) at 1000 ppm. In addition, the height of corn plants (*Zea mays*) decreases by 10% compared to the control at 25 ppm, this decrease reaches about 30 to 50% at 1000 ppm (Stojanovic *et al.,* 2010).

Effect of uranium on the radicles length of *Cleome ambryocarpa*: After 15 days of exposure of *Cleome amblyocarpa* seeds to increasing concentrations of U (Fig. 6), the measurement of radicles lengths reveals that the 50 and 100 ppm of U doses stimulate radicles elongation compared to the control with values of 3.91cm and 4.91cm respectively. A control seed value estimated at 2.53 cm is recorded. From 150 ppm of U, there is a very significant decrease with a probability ($P<0.001$) for doses of 150, 200, 250 and 300ppm of U, the root lengths are evaluated respectively at 3.78, 2.67, 1.88 and 1.11cm.

Effect of uranium on the kinetics of the radicles length of *Cleome ambryocarpa*: The kinetics of radicles

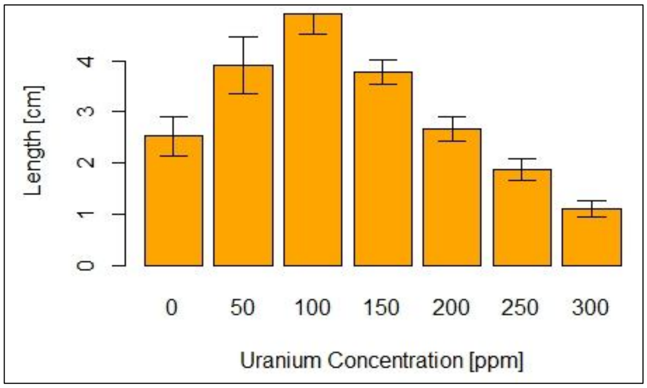

Fig. 6: Effect of uranium on the radicles length of *Cleome amblyocarpa* seedlings.

elongation of *Cleome amblyocarpa* seedlings treated with 50, 100 ppm of U concentrations is faster than the control. In contrast, in seedlings treated with 200, 250 and 300 ppm of U, kinetics are slower and the final length of the radicles is shorter. Elongation begins on the 3rd day and continues until the 11th day with constant values (Fig. 7).

Uranium impacts the radicles growth of *Cleome amblyocarpa* seedlings by stimulating root elongation relative to the control and significantly reduction radicles length as from 100ppm of U concentration. Last year Jing *et al.,* (2018) showed that the lower U concentration in sandy loam has promoting effect on seed germination and root growth of tomato, cucumber, kohlrabi, radish, cabbage and spinach. When the concentration of U increases more than the maximum limit of the tolerance of seedlings, the metabolism of plants will be disturbed, the absorption and transportation of some mineral elements are hindered, resulting in the decrease of root length. This result is similar to the effects of U on root length of sun-flower (Jagetiya and Purohit, 2006). Uranium concentrations have been shown to range from 0.1 to 4 µM, stimulating root biomass production compared to the control in beans (*Phaseolus vulgaris*). The percentage of root elongation was up to 7 times higher at 87 nM

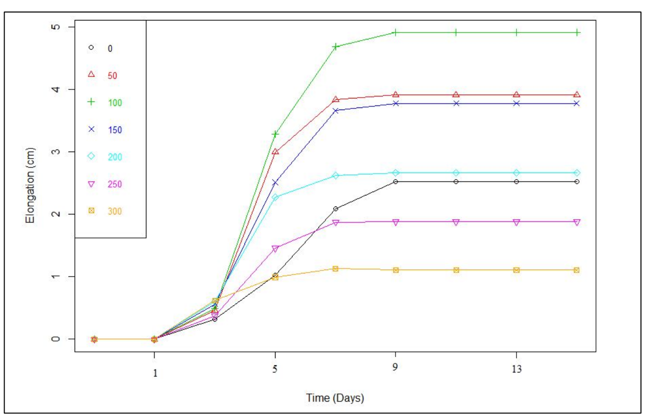

Fig. 7: Effect of uranium on the kinetics of the radicles length of *Cleome amblyocarpa.*

uranium than that of the control (Laroche, 2005).

Panda *et al.,* (2001) showed that the presence of uranyl nitrate in hydroponic solution at a concentration of 25 µM significantly inhibits root growth and cell division of onion bulbs (*Allium cepa*) after 5 days of exposure. In a study on peas (*Pisum sativum*), Ebbs *et al.,* (1998a) found uranium toxicity on roots exposed to 5 µM for 7 days: blackening of root apexes, j" torsion k" of lateral roots, as well as a decrease in root biomass by a factor of 2 to 3. In beans (*Phaseolus vulgaris*), root yellowing is retained after 7 days exposure to 1000 µM (Vandenhove *et al.,* 2006). Few studies have been conducted on the toxicity of uranium to root elongation, but the literature agrees that high uranium concentrations severely inhibit the development of roots in higher plants. Old studies, dating back to the beginning of the last century, have shown that small amounts of uranium can stimulate plant development, both at the root and aerial levels. (Stoklasa and Penkava, 1928).

The phenomenon of stimulation at low doses and inhibition at higher doses is found under the generic name of hormesis (from the Greek Hormæin, exciter). The hormesis effect could be transient and there are still uncertainties about how to interpret this phenomenon, from the perspective of the biochemical mechanisms involved. Hormesis would be a consequence of an adaptive response common to biological systems to the inhibitory effects that toxic agents have in common at higher concentrations (Stebbing, 1998). Finally, various authors have concluded that a root elongation test is valid and sensitive for testing the toxicity of heavy metals in the environment and in contaminated soils (Wong and Bradshaw, 1982, Ratsch and Johndro, 1986).

Conclusion

The germination evaluation shows that uranium affects the germination parameters examined in *Cleome amblyocarpa* seeds. By stimulating the germination capacity of the seeds and the development of the radicles. Increasing concentrations of U up to 300ppm result in a decrease in the aerial elongation of seedlings relative to the control. Taking into account all the germination parameters studied, *Cleome amblyocarpa* seeds were more tolerant to the metallic stress caused by U stress. It would be advisable to make histological sections at the radicle level.

Acknowledgements

I would like to thank Mr. Reguieg Yassaad Houcine Abdelhakim responsible for our doctoral project, Mr. Nourri Tayeb and Mr. Ferrah Nacer of Naama University who provided me with the seeds and uranyl nitrate solution. I would also like to express my gratitude to Mr. Benaichata Lazreg, Professor at the University of Tiaret, for teaching us the R software. Thank you to Mrs. Neggaz Nour Elhouda.

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